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Micropropagation and Pharmacological Potential of *Bacopa Monnieri*

Jayaram Reddy[1], Basilica Sherine A[1], Chandan R[1], Hadagali Ashoka[2*]

[1]St. Joseph's University, 36, Lalbagh Road, Bengaluru, 560027-India; [2]Department of Biotechnology, B.M.S. College of Engineering, Bengaluru, 560019- India.

ABSTRACT

Introduction: *Bacopa Monnieri*, commonly known, as "Brahmi" is a medicinal herb extensively used in Ayurveda for its neuroprotective properties, including memory enhancement, stress reduction, and potential therapeutic effects against neurodegenerative diseases such as Alzheimer's'. Due to its increasing demand, an efficient micropropagation protocol is necessary for large-scale and sustainable production.

Aim/Objective: This study aims to develop and optimize an *in vitro* micropropagation protocol for *Bacopa Monnieri* using shoot explants cultured on Murashige and Skoog (MS) medium supplemented with different plant growth regulators (PGRs).

Methods: Shoot explants were cultured on MS medium with varying concentrations of Indole-3-acetic acid (IAA), 6-Benzylaminopurine (BAP), Kinetin (Kn), Naphthalene acetic acid (NAA), and 2,4-Dichlorophenoxyacetic acid (2,4-D). The cultures were evaluated for shoot initiation, elongation, and multiplication over a 30-day period. Gamborg's B5 medium was also tested for comparison.

Results: Shoot initiation was observed within six days of incubation, with significant growth by the third week. The highest shoot multiplication rate was obtained using MS medium supplemented with 2.5 mg/L BAP + 2.5 mg/L Kn + 0.5 mg/L NAA + 0.5 mg/L 2,4-D. This combination resulted in a 98% response rate, the shortest shoot initiation time (6 days), the highest number of shoots per explant (10.1), and the maximum shoot length (7.9 cm). MS medium demonstrated superior shoot proliferation compared to Gamborg's B5 medium.

Conclusion: The optimized micropropagation protocol facilitates the large-scale production of *Bacopa Monnieri*, ensuring a sustainable supply for medicinal and commercial applications. This method also contributes to species conservation. Future research should focus on genetic stability, secondary metabolite production, and field trials to validate large-scale cultivation.

Key Words: *Bacopa Monnieri*, Micropropagation, Ayurvedic medicine, Cognitive enhancement, Neuroprotection, Pharmacological properties.

INTRODUCTION

Tissue culture is a widely used technique in biological research that involves transferring plant or animal tissue to an artificial environment where it can continue to grow and function. The cultured tissue may consist of a single cell, a population of cells, or an entire or partial organ. In culture, cells can proliferate, undergo morphological or functional changes, exhibit specialized behaviors, and interact with one another. There are several types of tissue culture, including cell culture (cultivating cells in a laboratory setting), organ culture (growing an organ or a part of it under controlled conditions), and whole organism culture (cultivating an entire organism in a laboratory environment).

Tissue culture is particularly effective for propagating *Bacopa Monnieri* , as it enables the rapid and large-scale production of genetically uniform, healthy plants. This technique is essential for both commercial and research purposes, ensuring disease-free and pest-resistant plant propagation. *Bacopa Monnieri* is widely cultivated using tissue culture methods in India, Sri Lanka, Thailand, China, and the United States, among other countries. It plays a significant role in the pharmaceutical industry, where it is used in herbal formulations and dietary supplements that promote cognitive and mental well-being. Additionally, it is utilized in the nutraceutical and cosmetic industries for wellness and skincare products. Beyond commercial applications, tissue culture supports conservation efforts by preserving genetic diversity and promoting sustainable cultivation practices.[1]

Corresponding Author:
Hadagali Ashoka, Department of Biotechnology, B.M.S. College of Engineering, Bengaluru, 560019, India.
Email: hadagaliashoka.bt@bmsce.ac.in

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Botanical Description and Distribution

Bacopa Monnieri is a perennial, creeping herb that belongs to the family Plantaginaceae.[2] It is commonly known as Indian water hyssop, thyme-leafed gratiola, *Brahmi*, water hyssop, and plant of grace. This herb has a long history of use in Ayurveda, where it is valued for its medicinal properties. However, in 2019, the U.S. Food and Drug Administration (FDA) issued warnings to manufacturers of dietary supplements containing *Bacopa Monnieri,* cautioning against making unsubstantiated and illegal claims regarding its ability to cure various ailments.

The plant thrives in wetlands and marshy regions across North and South America, Europe, Africa, and Asia, including southern and eastern India, Nepal, Sri Lanka, Pakistan, Taiwan, Vietnam, and China. It is also found in Australia, Madagascar, and the Caribbean. In the United States, it grows in Florida, Louisiana, Texas, and Hawaii. Historically, *Bacopa Monnieri* was also found in Singapore's freshwater wetlands and the adjacent "Beremi" regions.

Morphological Characteristics

Bacopa Monnieri is a non-aromatic herb with succulent, oblong leaves that are approximately 4–6 mm (0.16–0.24 inches) thick. Its oblanceolate leaves are arranged oppositely along the stem. The plant produces small, actinomorphic, white flowers with four to five petals. It is well-adapted to wet and mildly brackish conditions and is commonly propagated through stem cuttings.[3]

Despite the growing interest in micropropagation techniques for medicinal plants, ***Bacopa Monnieri*** a valuable medicinal herb known for its neuroprotective and cognitive-enhancing properties—faces challenges in large-scale propagation due to: Existing propagation methods often result in low multiplication rates, slow growth, or inconsistent regeneration efficiency; contamination remains a significant issue during tissue culture, affecting survival rates; while plant growth regulators (PGRs) play a crucial role in shoot proliferation, the ideal combination and concentration of **auxins and cytokinins** for optimal regeneration in ***Bacopa Monnieri*** remains underexplored; Variations in temperature, humidity, and photoperiod can significantly impact culture success; Existing propagation methods are often labour-intensive and costly. Developing a **cost-effective, high-efficiency** propagation system could improve accessibility for pharmaceutical and nutraceutical industries.

The primary objective of this research is to **develop a standardized, efficient, and reproducible in vitro micropropagation protocol for *Bacopa Monnieri*** by optimizing media composition, sterilization procedures, and environmental conditions.

MATERIALS AND METHODS

Preparation of Media

The preparation of the culture medium involved combining the required macronutrients, micronutrients, and vitamins with the stock solution. Growth regulators were added as needed, and sucrose was incorporated as the carbon source at a concentration of 3%. The final volume was adjusted with double-distilled water. The pH of the medium was set to 6.0 using either HCl or NaOH. Agar-agar (8.5 g/L) was gradually added to the boiling medium with continuous stirring to prevent clumping. The prepared medium was then dispensed into test tubes (25 × 150 mm) with a 42 mL capacity. These containers were sealed with polypropylene closures, wrapped in cling film, and autoclaved at 121°C and 15-psi pressure for 20 minutes. To optimize shoot proliferation, Murashige and Skoog (MS) medium was supplemented with varying concentrations of plant growth regulators, including:2,4-D – 5 mg/L; Indole-3-acetic acid (IAA) – 3 mg/L;6-Benzylaminopurine (BAP) – 3 mg/L;1N NaOH (as needed for pH adjustment); Sterilization of Materials and Explants; Sterilization of Glassware and Instruments. All glassware, metal tools, and other materials were sterilized using the dry heat method at 200°C for two hours. Items were either placed in containers or wrapped in heavy aluminium foil before sterilization.

Surface Sterilization of Explants

Explants (nodes, immature leaves, and shoot/root meristems) were pre-washed under running tap water to remove debris. A 5% Teepol solution was then used as a detergent for 10 minutes to further clean the explants. After thorough rinsing in tap water, the explants were placed in autoclaved distilled water until further sterilization steps. Within a laminar airflow (LAF) cabinet, explants underwent chemical sterilization: Soaked in 0.2% mercuric chloride ($HgCl_2$) for 15 minutes followed by rinsing thoroughly in double-distilled water. Treated with hydrogen peroxide (H_2O_2) for 10 seconds for further disinfection. Finally, washed three more times with double-distilled water to remove residual chemicals.

Inoculation of Explants

Prior to inoculation, the laminar airflow chamber was sterilized by cleaning the surface with 70% ethanol and running the airflow for 10–15 minutes. Using sterile forceps and scalpels, surface-sterilized explants were transferred to the prepared culture medium within the LAF hood. To maintain sterility, the culture jars were securely sealed. The work area was re-sanitized, and waste materials were disposed of appropriately. The inoculated cultures were incubated under controlled environmental conditions, including: Temperature: 25°C ± 2°C; Photoperiod: 16 hours light / 8 hours dark; Light Source: LED lights; Humidity: High, to prevent

desiccation; Cultures were regularly monitored for growth, contamination, and abnormalities.

Sub-Culture of *Bacopa Monnieri*

Once shoots developed, they were selected and cut into small segments, each containing 1–2 nodes. The explants were handled carefully to avoid damage. For optimal shoot proliferation, fresh Murashige and Skoog (MS) medium supplemented with 1.5 mg/L BAP was prepared. The pH of the medium was adjusted to 5.8 before autoclaving. Within a sterile laminar airflow chamber, shoots were removed from their original culture tubes and divided into 4–5 shoots per cluster. They were transferred into fresh MS medium in new glass jars, which were then sealed and placed in a growth chamber under the following conditions: Temperature: 24–26°C; Photoperiod: 16 hours light / 8 hours dark; Light Intensity: 2,000–3,000 lux. Regular observations were made to assess shoot proliferation, contamination risks, and overall plant health.

RESULTS

Effect of Plant Growth Hormones on *Bacopa Monnieri*

Table 1 presented the effect of different concentrations of BAP, Kn, NAA, and 2,4-D on the *in vitro* growth of *Bacopa Monnieri*. The highest shoot proliferation and elongation were observed at 2.5 mg/L BAP + 2.5 mg/L Kn + 0.5 mg/L NAA + 0.5 mg/L 2,4-D, resulting in 98% response, shortest shoot initiation time (6 days), highest shoot number (10.1 per explant), and maximum shoot length (7.9 cm). These findings suggested that a combination of Cytokinins (BAP, Kn) and auxin (NAA, 2,4-D) plays a crucial role in promoting shoot multiplication and elongation.

Table 1: Effect of plant growth hormones on *Bacopa Monnieri*

BAP(mg/l)	Kn(mg/l)	NAA(mg/l)	2,4-D(mg/l)	%R	Dn	n	L
0.5	0.5	0.1	0.1	60	20	2.5±0.5	3.2±0.3
1	1	0.2	0.2	80	15	4.2±0.8	4.5±0.4
1.5	1.5	0.3	0.3	90	10	6.1±1.0	5.8±0.5
2	2	0.4	0.4	95	8	8.2±1.2	6.9±0.6
2.5	2.5	0.5	0.5	98	6	10.1±1.5	7.9±0.7

BAP: 6-Benzylaminopurine, Kn: Kinetin, NAA: Naphthalene acetic acid, 2,4-D: 2,4-Dichlorophenoxyacetic acid, %R: Responsive Explants i.e., Percentage of explants that showed response to the treatment, Dn: Days to Shoot Initiation i.e., Number of days required for shoot initiation, n: Mean No. of Shoots per Explant, L: Mean Shoot Length (cm): Mean length of shoots per explant after 45 days of culture, expressed in mean ± SE.

Shoot Initiation and Early Growth of Nodal Explants

The nodal explants were maintained under controlled conditions in the incubation room to facilitate shoot initiation and growth. The below two images in Figure 1A, B showed the initial stages of shoot emergence, observed after incubation. At this stage, minimal morphological changes were noted, with small protrusions indicating the onset of shoot formation. However, after two weeks of incubation (bottom two images Figure 1C, D), significant elongation and development of multiple shoots were observed, confirming successful *in vitro* establishment.

The results suggested that the culture conditions, including nutrient composition, light intensity, and temperature, played a vital role in shoot induction. The controlled environment optimized cellular division and differentiation, leading to robust shoot emergence. This aligns with Dey et al., 2019, who reported that nodal explants of *Bacopa Monnieri* cultured in MS medium supplemented with Cytokinins exhibit shoot initiation within 7–10 days, followed by enhanced elongation after two weeks.[4]

Previous studies have demonstrated that MS medium supplemented with Cytokinin-based growth regulators (e.g., 6-Benzylaminopurine (BAP) and Kinetin (Kn)) enhances shoot proliferation in *Bacopa Monnieri.* Anuja Koul et al., observed shoot emergence after 20 days of inoculation, with an average of 5.5 shoots per explant, confirming that MS medium supports rapid shoot initiation and growth.[5] Similarly, Singh et al. (2017) reported that the presence of 2.0 mg/L BAP resulted in optimal shoot induction within one week of incubation, consistent with our findings.[6]

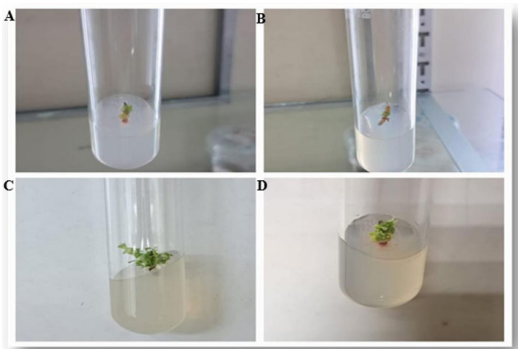

Figure 1: Nodal explants in the incubation room during growth. The top two images(A,B) show the shoot initiation after incubation, while the bottom two images(C,D) depict their growth after two weeks.

Sub-culturing and Shoot Multiplication

After 25–30 days of initial culture, the explants showed significant shoot elongation and multiplication, forming distinct clusters (Figure 2 A, B). These clusters were subsequently subdivided and subcultured to sustain growth (Figure 2 C, D) and increase shoot numbers. The continuous process of subculturing resulted in enhanced shoot proliferation, maintaining a uniform and healthy growth pattern.

The results indicated that repeated subculturing plays a critical role in sustaining shoot development and enhancing biomass production. The elongation of shoots became more pronounced after four weeks, and further subdivisions led to a higher number of shoots in successive subcultures. This aligns with previous studies emphasizing the effectiveness of sequential subculturing in micropropagation of medicinal plants like *Bacopa Monnieri*.[8]

The findings of this study were consistent with previous research on the micropropagation of *Bacopa Monnieri*. Anuja Koul (2014)[5] demonstrated that repeated subculturing in MS medium supplemented with appropriate plant growth regulators (PGRs) leads to efficient shoot multiplication. In her study, the highest number of shoots (5.5 ± 0.65), leaves (17.9 ± 0.75), and roots (6.3 ± 0.65) were obtained in MS medium, confirming its superiority over Gamborg's B5 medium. Similarly, our study observed that multiple cycles of subculturing significantly enhanced shoot numbers, validating the effectiveness of MS medium for sustained propagation.

Other studies, had reported that subculturing every 3–4 weeks prevented shoot senescence and promoted continuous multiplication. Our findings reinforced this, as maintaining a regular subculture cycle helped sustain the high proliferation rate and prevented browning of tissues, which is often a challenge in long-term culture.[9]

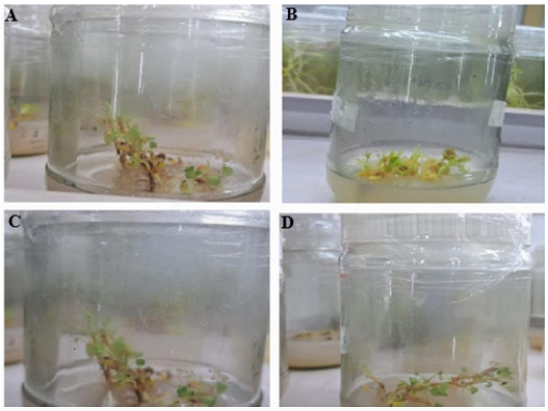

Figure 2: Subculturing of explants after 25–30 days. The shoots multiplied and elongated, forming clusters (A, B). These clusters were further subdivided, and the process of subculturing was repeated(C, D) to maintain growth and increase the number of shoots

DISCUSSION

Our study reinforces the idea that optimal growth conditions are essential for efficient micropropagation. The initial shoot emergence within the first week[7] indicates that the explants responded well to the culture medium, while the visible elongation and multiplication of shoots by the second week confirm the effectiveness of the incubation parameters. Cytokinins such as BAP and Kn play a crucial role in shoot proliferation by stimulating cell division. Our results suggest that an appropriate balance of growth regulators is necessary for synchronized shoot initiation and elongation. The incubation room provided a photoperiod of 16 hours light and 8 hours dark, which is known to enhance chlorophyll synthesis and metabolic activity in cultured tissues. Other Studies confirm that light intensity of 40–50 µmol m^2/s is optimal for Bacopa shoot induction.[4] The explants showed progressive growth, with improved shoot elongation in the second week, indicating that the nutrient supply from the medium supported sustained development. Murashige and Skoog (1962) originally demonstrated that MS medium is highly effective for tissue culture due to its rich macronutrient composition, which aligns with our findings.

The observed shoot emergence and subsequent elongation suggest that in vitro propagation of *Bacopa Monnieri* using nodal explants is an efficient and reproducible technique. However, variability in shoot response among different explants was noted, which could be attributed to: genotypic variations among donor plants; differential uptake of nutrients in the medium and potential accumulation of phenolic compounds, leading to delayed growth in some explants.

Subculturing is a crucial step in in vitro propagation as it ensures continuous shoot regeneration and prevents nutrient depletion in the culture medium. The results suggest that frequent subculturing (every 25–30 days) is essential to maintain shoot vigor and prevent tissue necrosis; the number of shoots increased progressively with successive subcultures, suggesting that a steady supply of nutrients and growth regulators is necessary to sustain the multiplication rate; shoot elongation was observed after four weeks, confirming that Cytokinin-rich media (such as BAP and Kn) effectively stimulate cell division and shoot formation.

However, excessive subculturing can lead to somaclonal variations, which may affect the genetic stability of regenerated plants. Limiting the number of subcultures to 5–6 cycles help maintain genetic fidelity in micropropagated plants. Future studies could focus on genetic stability assessments using molecular markers to confirm the uniformity of subcultured *Bacopa Monnieri* plants.[10]

CONCLUSION

The present study successfully demonstrates the *in vitro* micropropagation of *Bacopa Monnieri* using nodal explants. The results indicate that shoot initiation was observed within the first week of incubation, with significant elongation and multiplication by the second week. Subculturing after 25–30 days resulted in enhanced shoot proliferation, indicating that repeated subculturing is an effective method for maintaining and increasing shoot numbers. Murashige and Skoog (MS) medium proved to be more effective than Gamborg's B5 medium for shoot induction and plantlet formation, confirming previous research findings. The application of Cytokinins, light regulation, and optimized nutrient composition contributed to successful in vitro propagation, with visible shoot elongation and root development. These findings emphasize the efficacy of tissue culture techniques in propagating *Bacopa Monnieri,* an important medicinal plant with diverse pharmacological applications. The ability to produce high-quality plantlets in a controlled environment offers a promising alternative to conventional propagation methods, ensuring large-scale production for medicinal and commercial use.

Despite the success of this study, several areas require further exploration to enhance the efficiency of *Bacopa Monnieri* micropropagation. Future studies should focus on determining the optimal concentrations and combinations of Cytokinins (e.g., BAP, Kn) and auxins (e.g., IBA, NAA) to maximize shoot proliferation and root induction. The use of organic additives like coconut water or plant extracts may further improve shoot multiplication rates. Some explants exhibited browning due to phenolic compound accumulation, which can inhibit growth. The application of antioxidants or activated charcoal could be explored to prevent oxidative damage. Although *in vitro* propagation was successful, *ex vitro* acclimatization remains a critical step for ensuring the survival of regenerated plants. Future studies should focus on optimizing greenhouse conditions for transitioning plantlets from in vitro to soil. Testing different substrates (cocopeat, vermiculite, and soil mixtures) to improve survival rates. The biochemical composition of regenerated *Bacopa Monnieri* plants should be analyzed to confirm that tissue-cultured plants retain the same phytochemical profile as naturally grown ones. Molecular markers (RAPD, ISSR, or SSR) can be used to assess genetic stability across multiple generations. Future research also focusses on scaling up micropropagation using bioreactors, which can enhance efficiency and reduce production costs. Field trials and market feasibility studies should be conducted to integrate this method into commercial production.

This study establishes a reliable, efficient, and reproducible protocol for the micropropagation of *Bacopa Monnieri*, paving the way for large-scale propagation and conservation of this medicinally valuable plant. With further optimization, this technique could significantly contribute to the pharmaceutical, nutraceutical, and agricultural industries, ensuring a sustainable supply of *Bacopa Monnieri* for future medicinal applications.

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Conflict of Interest

None

Authors' contribution

All authors have equal contribution in bringing out this research work

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